

Partial Characterization of Human Neutrophil Plasma Membrane Components which Bind Concanavalin A and Wheat Germ Agglutinin¹

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ABSTRACT. Partial characterization of normal human polymorphonuclear neutrophil (PMN) plasma membrane components which bind Concanavalin A (Con A) and wheat germ agglutinin (WGA) was accomplished. Plasma membrane preparations of PMNs, separated on 9% SDS-polyacrylamide (PAGE) gels, showed the presence of 24 protein bands after Coomassie blue staining. Lactoperoxidase-catalyzed cell surface radioiodination showed that 16 of these bands represented cell surface proteins containing tyrosine residues. Molecular weights (MW) of these proteins ranged from 32,000-300,000 daltons. The PMNs were also labeled with ³H-WGA and ³H-Con A in separate experiments. Electrophoretic separation of plasma membrane components and subsequent fluorography revealed three radioactive bands in ³H-WGA labeled gels (MWs of 90,000, 55,000, and 40,000) and two in ³H-Con A labeled gels (MWs of 70,000 and 34,000). The WGA/Con A labeling ratio was approximately 2:1. These labeled bands represented surface glycoprotein WGA and Con A receptors of PMN plasma membranes. Spectrophotometric comparison of ³H-WGA and ³H-Con A labeled bands indicated separate and distinct labeling of surface glycoproteins by WGA and Con A. These substantially different binding patterns indicate major differences in membrane carbohydrate residues that are sterically available for WGA and Con A binding.

OHIO J. SCI. 86 (3) 085-089, 1986

INTRODUCTION

Many external membrane proteins of mammalian cells are linked with various carbohydrate (CHO) residues which play a major role in cell membrane structure and function. These glycoproteins have been implicated in processes such as cell motility (Edelman 1976); cell metabolism (Baehner and Boxer 1979, Goldstein and Weissman 1979, Fearon 1980); membrane transport (Baehner and Boxer 1979); antigenicity (Rambourg 1971, Sharon 1979); cell surface receptors for viruses, bacteria, drugs (Edelman 1976); cell:cell recognition, contact inhibition, and malignant transformation (Edelman 1976); and cell:cell adhesion and communication (Rambourg 1971, Gahmberg and Hakamori 1973, Sharon 1979). Type and orientation of carbohydrate residues depend on specific function and metabolism of the cells (Rambourg 1971, Gahmberg and Hakamori 1973, Sharon and Lis 1975, Anderson and Gahmberg 1978, Parodi and Leloir 1978, Nicola, et al. 1980).

Lectins have been used extensively to investigate membrane structure and function and to isolate specific membrane constituents. They are a class of carbohydrate-binding proteins that were identified originally in plant extracts (Sharon and Lis 1975) as agglutinins of erythrocytes. They have two critical properties: 1) specificity for particular sugar residues and 2) bivalency or polyvalency (Barondes 1984). They are site-specific labels directed to specific oligosaccharide conformations. Use of a wide variety of lectins with different sugar specificities facilitates the localization of different specific oligosaccharide residues on the cell surface and has contributed much to the current concept of membrane structure.

Lectins are excellent surface probes since they attach reversibly to cells and do not enter cells during short periods of contact (Sharon and Lis 1975). They selectively

bind specific terminal CHO residues. The two used in this study were wheat germ agglutinin (WGA) and concanavalin A (Con A).

Wheat germ agglutinin has a molecular weight of 36,000 and is composed of two identical chains, each with two identical binding sites (Nicolson 1973, Ghavanandan and Katlec 1979). It binds terminal carbohydrate residues of N-acetyl-D-glucosamine (Nicola et al. 1980), N-acetyl-D-galactosamine and N-acetylneuraminic acid (Fairbanks et al. 1971, Sharon and Lis 1975, Burridge 1976, West et al. 1978) and various hybrid-type glycopeptides with N-acetylglucosamine residues (Cummings and Kornfeld 1982). Concanavalin A is a globular hemagglutinating protein with a molecular weight of 108,000 (Nicolson 1973, Hardman 1978). It has a tetrameric form with four equivalent binding sites (Nicolson 1973, Lee et al. 1978) and selectively binds terminal non-reducing residues of α -D-glucopyranisyl, α -D-mannopyranisyl, α -D-fructofuranosyl (Sharon and Lis 1972, 1975, Stobo and Rosenthal 1972, Burridge 1976) and various high mannose type glycopeptides including glucosylated, phosphorylated, and certain hybrid-type glycopeptides (Cummings and Kornfeld 1982).

Similarity of binding may be due to: 1) a common set of glycoproteins or glycolipids recognized by these lectins, or 2) a common glycosylation sequence of different glycoproteins and glycolipids on the cell surface (Nicola et al. 1980). Varying degrees of binding to different cells may reflect the relative abundance of common glycoproteins on different cells or reflect relative activities of glycosyltransferases on different glycoproteins (Bell 1978, Hoffman and McMahon 1978). Available evidence suggests both contribute to binding (Nicola et al. 1980).

In view of the important role of the PMN in protective functions against infective organisms and toxic absorption, knowledge of the type of proteins that make up receptor sites in its plasma membrane is basic for the understanding of how PMNs function in normal and pathological states. This study was designed to in-

¹Manuscript received 16 October 1985 and in revised form 17 March 1986 (#85-50).

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investigate some of these receptor sites in an attempt to elucidate, in part, the biochemical makeup of the PMN plasma membrane.

MATERIAL AND METHODS

Seligman's Balanced Salt Solution (SBSS) and Dulbecco's Phosphate Buffered Saline (DPBS) were purchased from Grand Island Biological Company (Grand Island, NY). Bromphenol Blue, dextran (MW 200,000-275,000, clinical grade), dimethyl sulfoxide (DMSO), 2,5-diphenyloxazole (PPO), glycine, histopaque, molecular weight markers (MW 16,000-280,000), phenylmethyl sulfonyl fluoride (PMSF), and sodium dodecyl sulfate (SDS) were obtained from Sigma Chemical Company (St. Louis, MO). All acrylamide gel components were purchased from Bio Rad Laboratories (Richmond, CA). Lactoperoxidase was obtained from Calbiochem (La Jolla, CA) and Nonidet P40 (NP 40) from BDH Chemicals (Poole, England). New England Nuclear (Boston, MA) supplied Na^{125}I (Sp. Act. 551.191 mCi/ml), ^3H -Con A (Sp. Act. 25.0 Ci/mM), and ^3H -WGA (Sp. Act. 60.0 Ci/mM).

Blood (50 cc) obtained from 10 healthy male (6) and female (4) human volunteers was mixed with 0.5 ml heparin (0.1 ml/10 cc blood) and swirled gently to prevent coagulation. The blood was diluted 1:2 with 1X SBSS, layered onto a histopaque cushion, and centrifuged for 25 min at 400g. The red blood cell-PMN pellet was resuspended with 30 ml SBSS and 5 ml of 4% dextran (1 ml/10 ml original blood volume) and incubated 60 min at 37°C. The supernatant containing PMNs was drawn off and pelleted (10 min, 400g). Red blood cell contaminants were shock-lysed with distilled water.

The final PMN pellet was resuspended in 0.02 M PBS (pH 7.3), to which 0.5 ml lysing solution was added. Lysing solution consisted of 0.5% NP 40, 2.0 mM PMSF, 150 mM NaCl and 50 mM trizma base (pH 6.8). The reaction mixture was incubated on ice for 40 min and then centrifuged 10 min at 400g to remove nuclei and cell debris. The supernatant was processed for PAGE.

The procedure for labeling cells with tritiated lectins was a modification of Nicola et al. (1980). The PMN pellet was resuspended in 0.1 ml of phosphate-buffered, saline-bovine serum albumin-sodium azide (PBS-BSA-azide) (0.02 M:1.00%:0.02%) (pH 7.3) to which 0.5 ml of tritiated lectin (WGA, 50 uCi or Con A, 35 uCi) was added. The reaction was allowed to proceed on ice for 25 min. After the solution was centrifuged for 10 min at 400g, the pellet was washed three times with 1.0 ml PBS (0.02 M, pH 7.3). Cells were then lysed and processed for PAGE.

Lactoperoxidase (LPO)-catalyzed cell surface iodination was performed essentially as described by Anderson and Gahmberg (1978). All reactions were done at room temperature. The PMN pellet was resuspended in 0.5 ml PBS (0.02 M, pH 7.3), and the following, in order, were added to initiate the reaction: 20 μl ^{125}I , 75 μl LPO (0.1%) and 20 μl hydrogen peroxide (0.001%). The reaction mixture was mixed well and incubated for 5 min, followed by the addition of 75 μl LPO and 20 μl hydrogen peroxide and another 5 min of incubation. Finally, 20 μl hydrogen peroxide were added followed by a 15 min incubation.

The cells were washed three times with 2 ml of cold DPBS (with intervening 10 min of centrifugation at 400g) to remove excess label. The final pellet was resuspended in 0.5 ml DPBS and 0.5 ml solubilizer, incubated on ice for 40 min, and centrifuged at 400g for 5 min to remove nuclei and cell debris. The supernatant was processed for PAGE.

Nine percent SDS-polyacrylamide slab gels were prepared according to a modified Laemmli and Eiserling (1968) procedure. Samples and cross-linked molecular weight marker proteins were electrophoresed at 40 V of constant voltage overnight. Gels were stained with Coomassie blue and either dried for autoradiography or processed for fluorography according to Bonner and Laskey (1974).

RESULTS AND DISCUSSION

Plasma membrane preparations of PMNs, separated on 9% SDS-PAGE gels, showed the presence of 24 protein bands after Coomassie blue staining (Fig. 1a). Twenty-two protein bands were seen in gels containing plasma membranes of radioiodinated PMNs (Fig. 1b). The number and positions of protein bands in the two gels were the same except for the absence of two bands in the lower part of the iodinated gels. There was only one very broad

and intensely stained band present. It is possible that three bands were present and migrated very close together and, therefore, were not resolvable.

The supernatant recovered after cell lysis was a whole cell preparation. Therefore bands visualized on PAGE represent proteins of plasma and granule membranes. However, lectin- and iodine-labeled bands represent proteins and/or glycoproteins of the plasma membrane since the label does not enter the cell during short periods of contact (Sharon and Lis 1975).

Lactoperoxidase (LPO)-catalyzed iodination of surface proteins is a semi-quantitative method of detecting cell surface proteins by labeling exposed tyrosine residues (Hubbard and Cohn 1976, Schmidt-Ulrich et al. 1976, Nigg et al. 1980). The high molecular weight of LPO precludes its entry into the cell; therefore, all labeled proteins are on the cell surface. Carrier-free ^{125}I was used to increase specific activity of binding (Hubbard and Cohn 1976, Schmidt-Ulrich et al. 1976, Nigg et al. 1980).

Radioautography of the iodinated gels determined that 16 bands were radioactive (Fig. 2). These bands represented cell surface proteins containing tyrosine residues. Molecular weights of these proteins and those of PMN plasma membrane gels ranged from 32,000 to 300,000, as determined by comparing their relative mobilities to those of molecular weight marker proteins.

Protein banding patterns of both ^3H -WGA and ^3H -Con A labeled PMN plasma membranes were essentially the same as the PMN plasma membrane banding pattern (Fig. 1a, 1c, 1d). Fourteen plasma membrane components from ^3H -WGA labeled PMNs were identified with Coomassie blue protein stain (Fig. 3). In PMN plasma membrane gels, the upper one-quarter of the gel contained bands (components 1-11) which stained faintly but were nevertheless distinct. The same area in ^3H -WGA labeled gels stained lightly with Coomassie blue. This indicated that protein was present in small amounts, but was beyond the detection limits of Coomassie blue stain. Thus, distinct bands were not seen. Molecular weights of the proteins in the ^3H -WGA labeled gels ranged from 32,000-185,000.

Twenty-five membrane components (MWs 32,000-300,000) from ^3H -Con A labeled PMNs were visualized with Coomassie blue stain (Fig. 4). Differences in staining intensities of some bands were observed between the lectin-labeled gels and PMN plasma membrane gels. These differences may have resulted from variations in proteolytic degradation of proteins, since the antiprotease (PMSF) used in the lysing solution does not completely prevent proteolysis (Amrein and Stossel 1980).

Fluorography of tritiated lectin-labeled gels revealed three radioactive bands in ^3H -WGA labeled gels (MWs 90,000, 55,000, and 40,000) and two (MWs 70,000 and 34,000) in ^3H -Con A labeled gels (Figs. 3, 4). The bands seen in ^3H -WGA labeled gels were separate and distinct from those seen in ^3H -Con A labeled gels (Fig. 5). Radio-labeled bands 16a and 21a (Fig. 3) found in ^3H -WGA labeled gels were absent in ^3H -Con A labeled gels (Fig. 4). Labeling density of the bands observed on x-ray films was much heavier in the ^3H -WGA labeled bands. When samples were corrected for dilution and differences in specific activity, the WGA/Con A labeling ratio was approximately 2:1. This agrees with

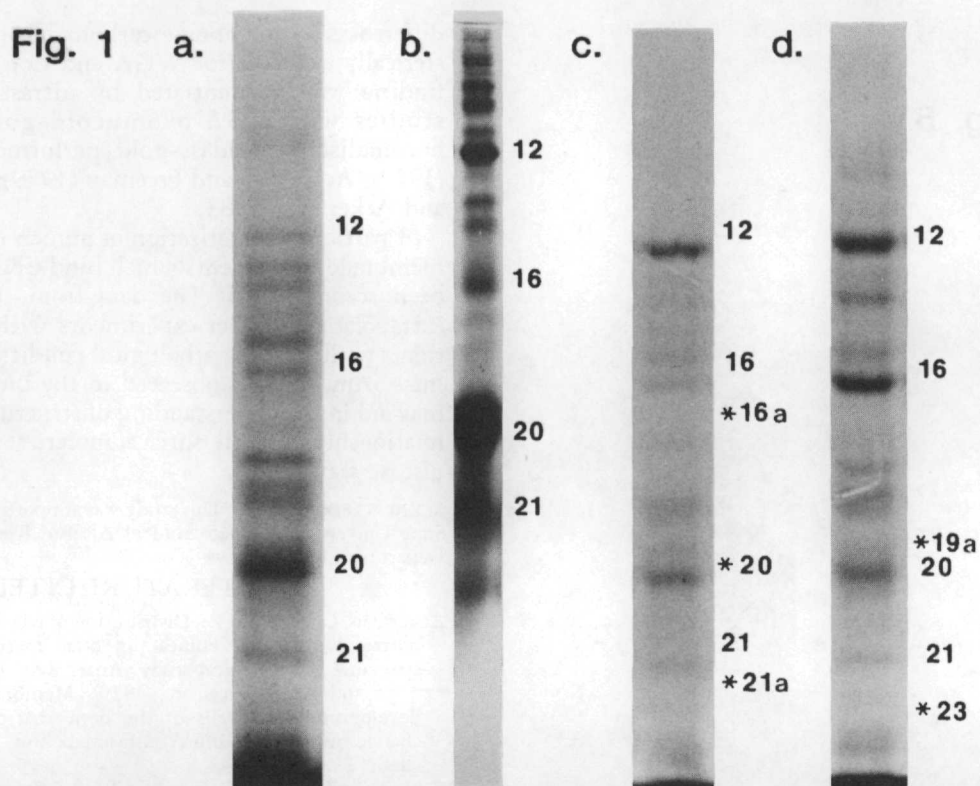


Fig. 2

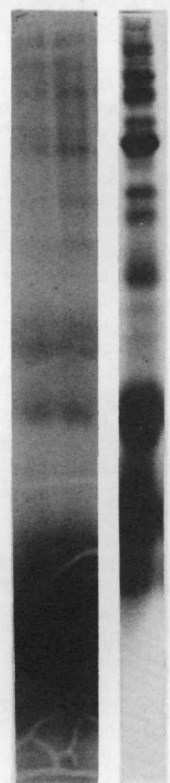


Fig. 3

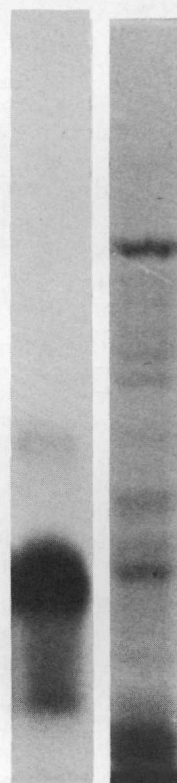


Fig. 4

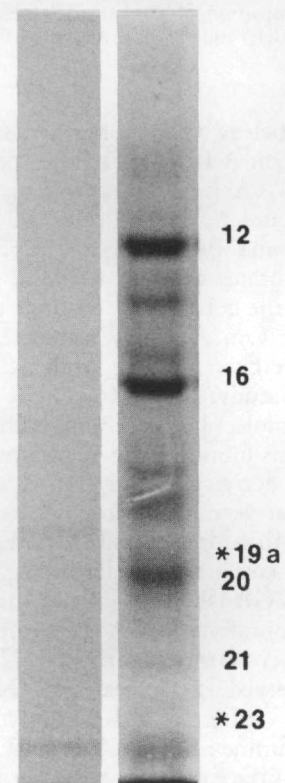


FIGURE 1. Banding patterns of Coomassie blue-stained PMN plasma membrane components separated on 9% SDS-PAGE gels: (a) untreated PMNs; (b) radioiodinated PMNs; (c) ^3H -WGA labeled PMNs; and (d) ^3H -Con A labeled PMNs. Numbers of components are indicated. (*Radioactive bands.)

FIGURE 2. Autoradiography pattern (left) of lactoperoxidase-catalyzed iodinated PMN cell surface proteins separated on 9% SDS-PAGE gels; (right) showing positions of radioactive bands.

FIGURE 3. Fluorography pattern (left) of ^3H -WGA labeled PMN surface glycoproteins separated on 9% SDS-PAGE gels; (right) showing position of radioactive bands (*).

FIGURE 4. Fluorography pattern (left) of ^3H -Con A labeled PMN surface glycoproteins separated on 9% SDS-PAGE gels; (right) showing positions of radioactive bands (*).

Fig. 5

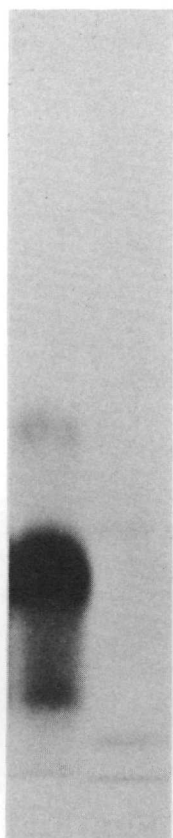


FIGURE 5. Comparison of position of labeled bands in ^3H -WGA labeled PMNs (left) and ^3H -Con A labeled PMNs (right).

the mean labeling values obtained from ultrastructural WGA and Con A labeling studies performed by Ackerman (1979), Ackerman and Freeman (1979), and Zinsmeister and Ackerman (1983).

Williams and Becker (1984) separated rabbit PMN plasma membranes on 9-15% PAGE gradient gels. They then labeled the gels with ^{125}I -Con A to find glycoprotein receptors for Con A. They found 12 such bands with molecular weights ranging from 38,000-220,000 daltons. In our study, only two Con A labeled bands were found with molecular weights which correspond to two of the proteins found in the Williams and Becker study. The specific activity of the Con A used in the present study was not very high, and the bands were very faint on the x-ray film. It is possible more labeled bands were present, but could not be detected.

The ^3H -WGA labeled bands in this study represented surface glycoprotein WGA receptors of PMN plasma membranes containing terminal N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, N-acetyl-neuraminic acid, and/or various hybrid-type glycopeptides with N-acetylglucosamine residues. The ^3H -Con A labeled bands represented surface glycoprotein Con A receptors of PMN plasma membranes containing terminal, non-reducing α -D-glucopyranosyl, mannopyranosyl, α -D-fructopyranosyl, and/or various high mannose-type and hybrid-type glycopeptide residues.

Comparison of ^3H -WGA labeled and ^3H -Con A labeled bands indicated separate and distinct labeling of surface glycoproteins by WGA and Con A. Spectrophotometric scans of films confirmed this. These substantially different binding patterns indicate major

differences in membrane carbohydrate residues that are sterically available for WGA and Con A binding. This finding was substantiated by ultrastructural binding studies with WGA-ovomucoid-gold and Con A-horseradish peroxidase-gold performed by Ackerman (1979), Ackerman and Freeman (1979), and Zinsmeister and Ackerman (1983).

A partial characterization of human neutrophil plasma membrane components which bind Con A and WGA has been accomplished. The data from this study can be extrapolated to other experiments with PMNs from patients with various pathological conditions such as leukemias. Any changes observed in the biochemical profiles may aid in the understanding of structural and functional relationships of cell surface moieties and their role in disease states.

ACKNOWLEDGMENTS. This study was supported in part by the Ohio State University Graduate Student Alumni Research Award and the Department of Anatomy.

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